Determination of Interaction Potentials of Amino Acids from Native Protein Structures: Tests on Simple Lattice Models.

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Abstract

We propose a novel method for the determination of the effective interaction potential between the amino acids of a protein. The strategy is based on the combination of a new optimization procedure and a geometrical argument, which also uncovers the shortcomings of any optimization scheme. The strategy can be applied on any data set of native structures such as those available from the Protein Data Bank (PDB). In this work, however, we explain and test our approach on simple lattice models, where the true interactions are known a priori. Excellent agreement is obtained between the extracted and the true potentials even for modest numbers of protein structures in the PDB. Comparisons with other methods are also discussed.

I. INTRODUCTION

The prediction of the three dimensional structures of the native state of proteins from the knowledge of their sequences of amino acids can only be achieved if the interaction potentials among various parts of the peptide chain in the presence of solvent molecules are known to some extent. Indeed, the native states of many globular proteins correspond to the conformations which are global minima of the free energy [1]. Thus the knowledge of the energy of a sequence in a given conformation would be an important step in the complete solution of this formidable problem, and also of the inverse one, i.e. the design of a sequence of amino acids that rapidly folds into a desired conformation.

A rigorous approach from "first principles", taking into account the quantum mechanics of the huge number of atoms constituting the protein is not practical and beyond actual possibilities.

An alternative approach consists of introducing a coarse-grained description mainly based on lattice models where the peptide chain is a self avoiding walk whose nodes represent extremely simplified amino acids. Models of this type have been widely used in the recent literature for various goals, ranging from folding dynamics to thermodynamic properties of folded states of proteins, see e.g. [2–4] .

One of the main difficulties with such simplified representations of the protein chain is the fact that an effective interaction Hamiltonian has to be used, which captures the essential features of the specific properties that one wishes to describe. For example, it is commonly believed that the native states of protein sequences ought to correspond to pronounced minima in conformation space [2]. In the most commonly used model Hamiltonian, "effective" two-body forces between neighboring (in space but not in sequence) amino acids are the only interactions that are considered [5]- [15]. These "effective" forces also take solvent induced interactions into account.

Traditionally, the potential energies of the interactions have been derived from pairing frequencies of amino acids observed in the native structures contained in the PDB [5–7,14]. The method, known as the quasi-chemical approximation, is widely used and relatively easy to implement in such a difficult context. In important recent work, Thomas and

Dill [14] have rigorously tested the underlying assumptions and approximations of the quasichemical method. Employing a lattice model with an a priori assigned interaction potential, one is able to construct a PDB identifying proteins as amino acid sequences having a unique ground state conformation (native state) among all possible conformations (this is accomplished by exhaustive exact enumeration for sufficiently small values of the protein chain length and/or the number of amino acid classes). Applying the quasichemical method to several of these exact cases, Thomas and Dill [14] demonstrated the inadequecies of the method and identified its possible weak points. Indeed, the interaction parameters could, in the worst cases, be off by as much as a factor of two and the native states of protein sequences of the PDB could be correctly identified onaverage in 84% of the cases which is poor, for the simple model employed.

Recently, we have proposed [16] a new optimization method for the determination of effective potentials – the derived potentials in the model considered by Thomas and Dill [14] are better than the ones obtained by the quasichemical method, but still do not match the true potentials. Nevertheless, these derived potentials allow 100% success in the prediction of the native structures! In this work we explain why this is so, and show that with the bare information contained in the native structures, no unique value of the candidate potentials can be given. Starting from a set of "good" sequences, i.e. sequences which have a unique ground state with the true potentials, their corresponding native structures, and a set of alternative structures, we can isolate a volume (cell) in the space of potentials. All points in this cell are equivalent to the true potential as far as the only requirement is that each good sequence has to recognize (i.e. has as the unique ground state) its native structure. The volume of the cell decreases as the protein chain length increases. In order to identify the most likely point around which the cell shrinks, we have to come up with some criterion. Here we propose a new implementation which leaves the success of good sequences in finding their own native states unaffected, and improves considerably the estimate of the extracted potentials.

As already stressed in [16], our method has its root in the original proposal by Crippen [9] but differs substantially in the implementation [10]. Our method is general, it can be implemented at any desired temperature T (lower than the minimum folding transi-

tion temperature of the good sequences we are considering), and it does not have any adjustable parameter. The method is explained in Sec. 2 , whereas Sec. 3 contains the results for a 2- and 4-class amino acid problem. Since our method is applicable in any spatial dimensionality, we have restricted ourselves to various checks in a two-dimensional square lattice with chains up to length 16 restricted to lie within a 5×5 square. A comparison of our results with those obtained using a recently proposed method of Mirny and Shakhnovich ([15]) is made in Sec. 4 .

II. THE MODEL

We consider a set of N_s sequences $\Omega_\sigma = \{\boldsymbol{\sigma}_s\}_{s=1,..,N_s}$ each comprised of N amino acids $\boldsymbol{\sigma}_s = \{\sigma_i^s\}_{i=1,..,N}$. Each sequence $\boldsymbol{\sigma}_s$ is postulated to have a unique native state (assumed to be the ground state and denoted by the superscript n) in a spatial conformation Γ_s^n that is known experimentally or otherwise. The corresponding set of native conformations is denoted by $\Omega_\Gamma = \{\Gamma_s^n\}_{s=1,..,N_s}$.

We assume that for a given number of amino acid types N_a, the effective interaction potentials can be written in the form of a symmetric interaction matrix $P_{\mu\nu}$, $\mu, \nu = 1, .., N_a$ and that similarly for each combination of a sequence and a conformation, a symmetric contact matrix $C(\Gamma, \boldsymbol{\sigma})_{\mu\nu}$, $\mu, \nu = 1, .., N_a$ is defined, giving the (effective) number of contacts between the different types of amino acids. The energy of a sequence $\boldsymbol{\sigma}$ in conformation Γ is thus given by

$$E(\Gamma, \boldsymbol{\sigma}) = \sum_{\mu \leq \nu = 1}^{N_a} P_{\mu\nu}\, C(\Gamma, \boldsymbol{\sigma})_{\mu\nu} \ . \tag{1}$$

There are a gigantic number of spatial conformations a sequence $\boldsymbol{\sigma}$ can take, which we label as $\Gamma_i(\boldsymbol{\sigma})$, and $\Gamma^n(\boldsymbol{\sigma}) \equiv \Gamma_0(\boldsymbol{\sigma})$ is the experimentally determined native state structure. At a temperature T, the probability that the sequence is in one of these conformations is simply given by

$$P_i(\boldsymbol{\sigma}) = \exp\left[-(E(\Gamma_i(\boldsymbol{\sigma}), \boldsymbol{\sigma}) - F(\boldsymbol{\sigma}))/T\right] \ , \tag{2}$$

where the Boltzmann constant k_B is defined equal to 1, and $F(\boldsymbol{\sigma})$ is the free energy, defined as

$$F(\boldsymbol{\sigma}) = -T \log \left(\sum_i \exp[-E(\Gamma_i(\boldsymbol{\sigma}), \boldsymbol{\sigma})/T] \right) \ . \tag{3}$$

Because the experimentally observed structure of the sequence is in the conformation $\Gamma^n(\boldsymbol{\sigma})$, the value of $P_0(\boldsymbol{\sigma})$ must be large ($> \frac{1}{2}$) at temperatures below the folding transition temperature. Indeed, $P_0(\boldsymbol{\sigma})$ should be equal to 1 at zero temperature, if the ground state is non-degenerate. In recent work, Crippen [11] has suggested that even with the knowledge of the exact contact potential from which the folding sequences and their unique native conformations are determined, one may not be able to correctly select which sequences fold to a desired target structure. The resolution [12] of this puzzle is that the right "score" to be maximized in the inverse folding problem is (2), i.e. $E(\Gamma(\boldsymbol{\sigma}), \boldsymbol{\sigma}) - F(\boldsymbol{\sigma})$ has to be minimized, and not just the energy $E(\Gamma(\boldsymbol{\sigma}), \boldsymbol{\sigma})$. A key feature of this score is that $F(\boldsymbol{\sigma})$, the free energy, does not depend on the specific target structure, but only on the sequence being considered. Thus, the determination of the exact contact potential is a valuable first step for an attack on the protein design problem, even though the currently used score needs to be modified.

In what follows, we describe a zero temperature version (which is appropriate in most instances) of such a procedure to extract the exact potentials. Furthermore, we restrict ourselves to models where each conformation is a self-avoiding walk whose elementary steps join nearest neighbor sites of a d-dimensional hypercubic lattice ($d = 2$ in the present applications). Amino acids are placed at the nodes of the visted sites, and contacts are defined between amino acids in neighboring sites but not next to each other along the sequence.

A. The Method

Instead of starting immediately with a cost function that has to be minimized, we concentrate for a moment on the space spanned by the interaction potentials.

Since all energies scale linearly with the amplitude of the interaction potentials, we have to keep e.g. the first parameter fixed ($P_{11} \to P_0$) to set a scale. Relabelling the remaining parameters $P_{\mu\nu} \to \vec{p} \equiv \{p_i\}_{i=1,..,N_p}$ ($N_p \equiv \frac{1}{2}N_a(N_a+1)-1$), and renumbering the contacts accordingly, we can rewrite (1) as

$$E(\Gamma,\boldsymbol{\sigma}) = \sum_{i=1}^{N_p} p_i c_i(\Gamma,\boldsymbol{\sigma}) + P_0 c_0(\Gamma,\boldsymbol{\sigma}) \equiv \vec{p}\cdot\vec{c}(\Gamma,\boldsymbol{\sigma}) + P_0 c_0(\Gamma,\boldsymbol{\sigma}) \ . \tag{4}$$

The fact that a sequence has a lower energy in its native conformation than in any alternative conformation, provides a linear inequality (or hyperplane) in the parameter space separating the space into allowed and forbidden halfspaces. We define:

$$I_i(\Gamma^{\rm alt},\Gamma^n_s,\boldsymbol{\sigma}_s) \equiv c_i(\Gamma^{\rm alt},\boldsymbol{\sigma}_s) - c_i(\Gamma^n_s,\boldsymbol{\sigma}_s) \ . \tag{5}$$

The allowed points in parameter space have to satisfy the linear inequality

$$\sum_{i=1}^{N_p} p_i I_i + P_0 I_0 \equiv \vec{p}\cdot\vec{I} + P_0 I_0 > 0 \ . \tag{6}$$

Repeating this operation for all the sequences in Ω_σ and for all the alternative conformations, and retaining only the allowed part of the parameter space that satisfies all the inequalities, we obtain a convex *cell* around the target parameters. This cell contains all the points that yield the correct native conformation as the unique ground state for each of the sequences in Ω_σ. In the test model, we have generated the set Ω_σ using the energy function (1), and therefore, the existence of the cell is guaranteed and the problem is well posed. For real proteins the form of the energy function is an ansatz that is tested by the (non)existence of a finite cell.

Each inequality corresponds to a hyperplane in parameter space separating allowed and forbidden half-spaces. The orientation of the hyperplane is given by $\vec{I}$, the offset from the origin by $P_0 I_0$. The distance of any point $\vec{p}$ in parameter space to this hyperplane, is related to the energy gap between the two configurations leading to this inequality (at the value of parameters given by $\vec{p}$) by the following linear equation:

$$\mathrm{d}(\vec{p},I) = \frac{\left|\vec{p}\cdot\vec{I} + P_0 I_0\right|}{\sqrt{\sum_i I_i^2}} = \frac{\mathrm{gap}(\vec{p},I)}{\sqrt{\sum_i I_i^2}} \ . \tag{7}$$

Using all the information in the data set, the cell is maximally reduced. A selection procedure is needed in order to isolate an optimal point within the cell. Instead of the cost functions used in [16], the optimal interactions are chosen such that the smallest gap among all the sequences in the training set is as large as possible. The cost function ($F_{\rm gap}(P)$) is hence taken to be minus the smallest gap, i.e.

$$F_{\rm gap}(P) = -\min_{\boldsymbol{\sigma}_s \in \Omega_\sigma} \min_{\Gamma \neq \Gamma_s^n} \{E(\Gamma, \boldsymbol{\sigma}_s) - E(\Gamma_s^n, \boldsymbol{\sigma}_s)\}$$
$$= -\left(\vec{p} \cdot \vec{I}(\Gamma^*, \Gamma_{s^*}^n, \boldsymbol{\sigma}_{s^*}) + P_0 I_0(\Gamma^*, \Gamma_{s^*}^n, \boldsymbol{\sigma}_{s^*})\right) \ , \qquad (8)$$

where $\boldsymbol{\sigma}_{s^*}$ and Γ^* are the sequence and the alternative conformation respectively that yield the minimum gap. To reiterate, the interaction potentials are chosen in such a way that the maximum minimum (mxm-) gap is obtained. For similar ideas see also [8].

This cost function has two major advantages over previous attempts. First, it automatically ensures that all sequences have their unique groundstates in the correct structures. In fact, a negative mxm-gap would imply that the a priori assumption of the form of the energy function (1) is incompatible with the data in the training set.

Second, it does not suffer from an unphysical bias due to statistical fluctuations that were present in the cost functions proposed in [16]. These cost functions not only make use of all inequalities, but also of the number of occurences of each inequality over the training set. Therefore, it may happen that inequalities that occur more frequently, push the optimal parameters away from their true values. One may expect all sequences in the training set to satisfy the minimimum conditions that make them good folders, which implies that each inequality is equally important, irrespective of the number of times it occurs. In realistic cases it may be important to rescale the energy gap associated with a given sequence with respect to its ground state energy. Because, in this work, we have sequences of the same length in a given training set, all ground state energies are roughly the same, and rescaling of the gap is not necessary.

Because the width of the energy gap is not equal to the distance in parameter space (7), inequalities that do not contribute to the boundaries of the cell may still influence the mxm-gap. Nevertheless, for all the cases that we have encountered, using only the inequalities contributing to the cell, we obtain exactly the same optimal parameters as the ones derived by using all inequalities. This facilitates our maximization procedure. The sequences have to be put on each alternative conformation only once before the optimization procedure, and we retain only those inequalities that contribute to the cell. Then we start our optimization procedure with only those few inequalities. Given the inequalities with respect to which to optimize, the design of an optimization procedure

is straightforward, because the gradient of each inequality is given by $\vec{I}$. Therefore, each step in the optimization procedure consists of the following replacement

$$\vec{p} \to \vec{p} + \gamma \vec{I}(\Gamma^*, \Gamma^n_{s^*}, \boldsymbol{\sigma}_{s^*}) \ , \text{ or} \tag{9}$$

$$\vec{p} \to \vec{p} + \gamma \vec{I}_{\min} \ , \tag{10}$$

where γ is a parameter that can be tuned to obtain fast convergence. Form (9) of the optimization algorithm is used when the equalities have to be recalculated putting each good sequence on the alternative conformations, while form (10) is used when the set of important inequalities is already known. In that case $I_{\min}$ is the inequality from this set that yields the minimum gap.

III. RESULTS

The method has been tested on models with an increasing number of interaction parameters to check the dependence on the dimensionality (N_p) of the parameter space. The test has been done on the normal H-P model [17], $N_a = 2$, with nearest neighbor (nn) interactions, i.e. with 2 free parameters ($N_p = 2$), and some variations like considering next to nearest neighbor interactions, to check the robustness of the method.
Furthermore, the method has been applied to models with 4 types of amino acids ($N_p = 9$) and nn interactions. For the latter we have also studied the dependence of the quality of the obtained parameters on the size of the PDB and of the set of alternative structures.

Although still feasible up to parameter numbers as high as $N_p = 9$, increasing the number of interaction parameters and thus the dimension of the cells, reveals the tendency that the advantage of putting the good sequences on the alternative structures only once, will be annihilated by having to calculate too many cornerpoints.
With increasing dimension, the number of inequalities contributing to the cell grows linearly, while the number of cornerpoints of the cell, however, grows exponentially. Therefore, one may have to opt for a hybrid method. The first step consists of a rough optimization recalculating the inequalities at each update (9). Once a point in parameter

space satisfying all constraints (i.e. in the cell) has been obtained, we select and save the inequalites which are at a distance less than some tolerance parameter from this point. The number of such inequalities is relatively low and it grows linearly with the number of parameters. Then, we fully optimize only with respect to these inequalities (10). An implementation of this method on the model with $N_p = 9$ for different choices of parameters shows that we roughly need 20-30 updates for the first step. Then, we need to typically save of the order of 100 inequalities to perform the second step.

This hybrid method is very efficient because it uses the insights in the geometry of the cells in parameter space and avoids unnecessary time loss due to exactly calculating the cell.

A. Results for the H-P model

In order to be able to compare our results with those of previous work, the first model, that we study extensively, is the H-P model introduced by Dill and co-workers [17], which has 2 types of amino acids. A contact is defined to be 1 for a nearest neighbour contact, as long as the two amino acids are next to each other along the sequence, and 0 otherwise. To fix the energy scale, we have choosen to fix the parameter ($E_{HH} \equiv P_0$). Hence, we are left with two independent parameters ($E_{PP} \equiv p_1$ and $E_{HP} \equiv p_2$) and a 2 dimensional parameter space, which allows us to clarify our reasoning with instructive pictures. We have considered three types of target interaction parameters: $(E_{HH}, E_{PP}, E_{HP}) = (-1, 0, 0),\ (-1, -1/\sqrt{2} \simeq -0.707, 0),\ (-2, -2, -1)$, and seven distinct groups of amino acid chains each of length: $N = 10, .., 16$.

In order to reduce the necessary computer time, we have taken all semi-compact 2 dimensional conformations on a square lattice. By semi-compact, we mean that we restrict the conformations to a box of size 5×5 (Tests with all conformations of a certain length on a square lattice show that the results are unaltered).

As alternative conformations, we have considered both the set of good conformations $\Omega_\Gamma^{\rm good}$ (having at least one sequence that has its unique native state in it), and the set of

all conformations $\Omega_\Gamma^{\rm all}$ (obtained by complete enumeration and also used to generate the good sequences). Although good results can be obtained considering only $\Omega_\Gamma^{\rm good}$, it is not excluded (and is indeed observed) that extra information can be gained (in the sense of new inequalities, further reducing the cell) by also considering new conformations from $\Omega_\Gamma^{\rm all}$. However, when the cells are closed, in all cases we obtain exactly the same set of optimized parameters. Since some inequalities are very rare, it is difficult to say how many alternative conformations are needed to maximally reduce the cell. Unfortunately, so far we have not found a criterion to determine beforehand whether a certain sequence and a given alternative conformation gives rise to a "tight" inequality (contributing to the boundaries of the final cell) or not. Therefore, although the obtained parameters are relatively stable to changes in the shape of the cell, the best strategy seems to be to use as much information as is available, or as is numerically feasible. It cannot be excluded that regenerating all the good sequences with the newly obtained parameters, would add some new "good" sequences to the set.

target	0.0	0.0				
N	E_{PP}	E_{HP}	truegap	mxmgap	vol($\Omega_\Gamma^{\rm all}$)	vol($\Omega_\Gamma^{\rm good}$)
10	/	/	1.0	/	0.0^o	2.034444^o
11	0.0	0.0	1.0	1.0	1.062500	0.643501^o
12	0.0	0.0	1.0	1.0	0.916667	0.643501^o
13	0.0	0.0	1.0	1.0	0.625000	0.0^o
14	0.0	0.0	1.0	1.0	0.444444	0.0^o
15	0.0	0.0	1.0	1.0	0.272817	1.203704
16	0.0	0.0	1.0	1.0	0.252315	0.611111

(3.1.a)

target	-0.707107	0.0				
N	E_{PP}	E_{HP}	truegap	mxmgap	vol($\Omega_\Gamma^{\rm all}$)	vol($\Omega_\Gamma^{\rm good}$)
10	/	/	0.12132	/	0.0^o	0.321751^o
11	-5/7	0.0-0.10	0.12132	0.142856	0.034722	0.034722
12	-5/7	0.0-0.04	0.12132	0.142856	0.017361	0.029514
13	-5/7	0.0	0.12132	0.142856	0.030556	0.030556
14	-5/7	0.0	0.12132	0.142856	0.015129	0.019097
15	-5/7	0.0	0.12132	0.142856	0.007955	0.007955
16	-5/7	0.0	0.12132	0.142856	0.007955	0.007955

(3.1.b)

target	-2.0	-1.0				
N	E_{PP}	E_{HP}	truegap	mxmgap	vol($\Omega_\Gamma^{\rm all}$)	vol($\Omega_\Gamma^{\rm good}$)
10	/	/	1.0	/	0.0^o	1.249046^o
11	-2.0	-1.0	1.0	1.0	0.733333	0.785398^o
12	-2.0	-1.0	1.0	1.0	0.733333	0.785398^o
13	-2.0	-1.0	1.0	1.0	0.900000	0.566729^o
14	-2.0	-1.0	1.0	1.0	0.733333	0.566729^o
15	-2.0	-1.0	1.0	1.0	0.357143	0.554762
16	-2.0	-1.0	1.0	1.0	0.215320	0.334641

(3.1.c)

From the tables 3.1.a, 3.1.b, 3.1.c and Fig.1, we see that the volume of the cells tends to decrease montonically with increasing sequence length. The only exceptions are observed with length $N = 13$, but are probably due to finite size effects. In two cases, a segment of a line of points in parameter space yields the same mxm-gap.

In all the cases that we have considered, and where the ratios of the target potentials are rational numbers made up out of small enough integers, the maximization of the minimum gap renders the exact potentials. Furthermore, we observe that all the obtained parameters are rational, even if the target parameters are not, due to the fact that in our model only an integer number of contacts is possible. It also explains the fact that for the target parameters $(-1, -1/\sqrt{2}, 0)$, the obtained parameter E_{PP} is invariably $-5/7$

for all sequence lengths $N = 11, .., 16$ although the cell changes drastically. One would have to consider (much) longer sequences to get contact numbers high enough to generate a rational number closer to $-1/\sqrt{2}$. This insensitivity may be lifted in cases where the number of contacts is no longer integer, e.g. for real space proteins.

For this set of target parameters, we have also considered taking only those good sequences with a minimum gap larger than certain threshholds (i.e. 0.5 and 0.75), and although the obtained cells are larger (it scales with $(\text{mingap})^{N_p}$), the obtained parameters are unaltered untill the cell ceases to be closed.

To get an idea of the performance of the algorithm as the dimension of parameter space increases, we did some checks on the following variations:

-a model with $N_p = 3$, with 2 kinds of amino acids as before ($N_a = 2$), but including a next to nearest neighbor (nnn)

interaction for a H-P contact,

-a model with $N_p = 5$, with 2 kinds of amino acids ($N_a = 2$) and nn and nnn contacts,

-a model with $N_p = 5$, with 3 kinds of amino acids ($N_a = 3$) and only nn-contacts, and

- models with $N_p = 9$, with 4 kinds of amino acids ($N_a = 4$) and only nn-contacts, see Sec. 3.2 .

The quality of the obtained parameters is always as good as those shown in tables 3.1.a,3.1.b,3.1.c and does not depend on N_p.

To check the sensitivity of the method to a wrong choice of energy function, we have generated good sequences and structures using 6 interaction parameters ($N_p = 5$, both $N_a = 2$, nn- and nnn-contacts and $N_a = 3$, nn-contacts), and tried to satisfy all inequalities using fewer parameters, e.g. ignoring nnn H-P contacts. The method immediately indicated that the cell does not exist, and thus that the number of parameters was insufficient. On the other hand, putting in more free parameters than were used to generate the good sequences, the irrelevance of these parameters is immediately recognized and their obtained values are (very close to) 0 .

B. Results for the 4 amino acids problem

The $P_{\mu\nu}$ matrix has 10 independent parameters in this case. Our tests have been carried out for four different sets of parameter values where each parameter is generated independently from a Gaussian distribution with mean -2 and variance 1. The length of the chain is 14. For each set of parameters, we have generated a PDB of about 600 sequences and their corresponding (unique) native states. Furthermore, the sequences have an energy gap, Δ (the energy difference between the first excited state and the ground state) greater then 0.5. Indeed it is thought [2] that real proteins in order to have thermodynamical stability and short folding times should possess a pronounced global minimum on the potential surface. A comparison with one case where $\Delta > 2$ is also presented. The trial Hamiltonian is parametrized as the true one and we have chosen the energy scale by fixing to its exact value one of the most negative $P_{\mu\nu}$'s. The remaining 9 parameters are then determined maximizing the minimum gap using the method explained above. We have also verified that simulated annealing techniques are quite efficient for this case and give the same set of extracted potentials as the method used in §III A. Figures 2a,b,c and d show the extracted potentials versus the true ones. The extracted potentials are then tested for *new sets* of "good" sequences for each of the four cases to determine their ground state configurations over all possible self avoiding chains of length 14. For all the sequences in the PDB, we get full success (Figure 2). Indeed, since the maximun gap has been calculated on a restricted set of conformations there is no guarantee a priori that the good sequences used in the optimization procedure recognize their own native state among all possible conformations. Thus it is important to test the extracted potentials using a new independent set of good sequences. In all four cases that we studied, at most 2 out of 628 do not found their original native state. The percentages of the correct determination of the native states using the extracted potentials are indicated in the table.

parameter set	size of the PDB	success
1	628	99.7%
2	716	99.9%
3	840	100%
4	798	100%

(3.2)

We have tested the performance of the method as the size of the PDB is decreased. Figure 3 shows how the percentage of success depends on the number N of sequences contained in the PDB. Only three of the four cases are shown for clarity (the fourth case has the same behaviour as the other three). The minimum N used is 14. Note that full success is almost reached for $N \sim 200 - 300$. For the first set of potential parameters, we have also generated a PDB with an energy gap $\Delta > 2$. The results are shown in fig.3 , and saturation is reached at about $N \sim 100$.

IV. COMPARISON WITH OTHER METHODS

A. The quasi-chemical method

The quasi-chemical method [5-7] is widely used in various forms for obtaining the effective potential between aminoacids and to provide "scores" for candidate protein structures. Briefly, the procedure is as follows: from the databank, one compiles the probability density, $f_{A,B}(r)$, that two specific aminoacids are at a distance r from each other. This quantity is a normalized one, and takes into account how often the individual aminoacids appear in the data base. The basic idea is that if A and B like each other, they are more likely to be near each other compared to a random reference state of a non-interacting gas of aminoacids. Conversely, if A and B dislike each other, they avoid each other a bit more than what one would expect from random considerations. This idea is then quantified in the form

$$E_{A,B}(r) \propto -kT \ln[f_{A,B}(r)] \ . \tag{11}$$

Additional considerations pertaining to how far apart two aminoacids are along the sequence are sometimes introduced in order to build in the correct secondary structure.

The derived quantities such as $E_{A,B}$ are now interpreted as the energies of interaction between the aminoacid pairs and used for determining which structure among many alternatives yields the most favorable value of the energy. The results using this method as obtained by Thomas and Dill [14] are shown in table 4.1, and have to be compared with the corresponding cases in tables 3.1.a, 3.1.b,3.1.c obtained by our method.

True			TD test [14]			
E_{HH}	E_{HP}	E_{PP}	E_{HH}	E_{HP}	E_{PP}	success
-5	-4	-1	-5	-3.0	+0.8	74%
-5	-1	-2	-5	-1.1	-2.1	100%
-5	-5	-1	-5	-3.7	+1.4	84%
-5	-3	+1	-5	-2.6	+2.5	96%
-5	-3	-1	-5	-2.4	0.0	64%

(4.1)

The rationalization for deriving the interaction energy from the observed pairing frequency has been stated to be Boltzmann's principle and also has been called the Boltzmann device. Boltzmann statistics pertains to the occupation probabilities for the energy levels of an individual system. Thus, if a system can have energies E_0, E_1, E_2, etc., the probability that the system has an energy E_i is proportional to $\exp[-\frac{E_i}{kT}]$.

We repeat several observations made in Seno et al. [16]. (Thomas and Dill [14] have also presented an important critique of the quasichemical method.) First, the native structures of distinct sequences of aminoacids do not correspond to the excitations of a single system. Instead, each of the sequences is a separate system, whose native state structure is known from experiment. Thus, the basic premise of the method is wrong. Second, even making the assumption that Boltzmann statistics did hold, there is no simple relationship between the observed pairing frequency and the energy of interaction as envisaged by (11). The role of temperature in (11) is unclear, because the native states of each of the sequences correspond to their ground states or equilibrium states at $T = 0$. Third, the quasichemical method relies on a reference state – the observed pairing frequencies are compared to those expected in this reference state in order to determine whether

two amino acids like each other and by how much. Often, this reference state is chosen as a noninteracting gas made up of all the aminoacids constituting all the sequences with known native structure. This does not seem to have a physical basis, because the sequences are all distinct entities and do not originate from a common soup of aminoacids.

These difficulties with the quasichemical method, which were already partially recognized in the literature (see ref. [14] and references therein), are avoided in our strategy. The sequences whose structures are known are analogous to quenched variables in statistical mechanics while the conformations that a given sequence can adopt, are the analog of annealed variables. A thermodynamic average can be performed over the annealed variables but not over the quenched ones. We use Boltzmann statistics but for each sequence separately. We deal with the energies directly and not with a derived quantity such as the pairing frequency. Indeed, our strategy embodies the complete information in the system and, in principle, has information not only about pairing frequencies but also triplet and higher order correlations. Our method does not rely on a reference state and the role of temperature is well-defined.

B. Mirny and Shakhnovich's method

Recently, Mirny and Shakhnovich (MS) [15] have proposed to use the Z-score [18], which is a measure of how pronounced the energy minimum corresponding to the native state is, to carry out protein design. The Z-score is given by:

$$Z(\boldsymbol{\sigma}) = \frac{E(\Gamma^n_{\boldsymbol{\sigma}}, \boldsymbol{\sigma}) - \langle E \rangle}{\mathrm{var}(E)} \ . \tag{12}$$

where the average of E, $\langle E \rangle$, and its variance, $\mathrm{var}(E)$, are computed for a set of alternative (decoy) conformations. The method of [15] entails the minimization of the cost function (harmonic mean)

$$\langle Z \rangle_{\mathrm{harm}} = (\sum_{\boldsymbol{\sigma} \in \Omega_\sigma} Z(\boldsymbol{\sigma})^{-1})^{-1} \ . \tag{13}$$

For each conformation Γ of the ensemble Ω_σ, the average $\langle E \rangle$ and the variance $\mathrm{var}(E)$ are calculated in an ensemble of phantom conformations with the same number of residue-

residue contacts as in Γ and with the assumption that these contacts occur independently of each other. This approximation will be discussed further later on. One can repeat these calculations for our cases.

We have implemented this method using (12) and (13), calculating $\langle E \rangle$ and var(E) exactly for each sequence using the structures of the PDB.

In order to have a finite minimum of $\langle Z \rangle_{\rm harm}$, it is necessary to fix the variance, or equivalently one of the interaction parameters like we did. MS also fix the average potential, which requires more information, which, apriori, one does not have. In the case of many interaction parameters, however, this might not be so crucial. Furthermore, in their implementation, MS do not explicitly require that all the $Z(\boldsymbol{\sigma})$'s be negative, since $\langle E \rangle$ and var(E) are approximated. For the H-P model, we first require that $Z(\boldsymbol{\sigma}) < 0$ for all $\boldsymbol{\sigma}$ in the PDB, and then we minimize $\langle Z \rangle_{\rm harm}$.

true	-1.0	-0.707107	0.0			
N	E_{HH}	E_{PP}	E_{HP}	$\#_{tot}$	$\#_{wrong}$	success
12	-0.969868	-0.747827	0.010588	728	4	99.45%
13	-0.990930	-0.719754	0.003577	750	0	100.0%
14	-0.977091	-0.738392	0.008377	2005	26	98.70%
15	-0.963963	-0.755398	0.012254	4302	77	98.21%
16	-0.972729	-0.744113	0.009735	8892	151	98.30%

(4.2.a)

true	-1.0	-0.707107	0.0			
N	E_{HH}	E_{PP}	E_{HP}	$\#_{tot}$	$\#_{wrong}$	success
12	-0.655207	-0.330015	0.474504	728	124	82.97%
13	-0.841471	-0.317697	0.568875	750	198	73.60%
14	-0.745093	-0.317782	0.526300	2005	675	66.33%
15	-0.657765	-0.327509	0.477554	4302	1754	59.23%
16	-0.740636	-0.328271	0.517735	8892	3274	64.00%

(4.2.b)

For the 4 amino acid case, the minimization of (13) leads to spurious minima corresponding to $\sum_{\boldsymbol{\sigma}} Z(\boldsymbol{\sigma})^{-1} \simeq 0$, since both positive and negative $Z(\boldsymbol{\sigma})$'s appear. This happens

both with the exact $\langle E \rangle$ and var(E) , and with the approximations of MS [15].

Since the search of the parameter domain where all the $Z(\boldsymbol{\sigma})$'s are negative was impractical in this case, we have modified (13) to $\langle |Z| \rangle_{\rm harm} = (\sum_{\boldsymbol{\sigma}} |Z(\boldsymbol{\sigma})|^{-1})^{-1}$.

Tables 4.2.a,4.2.b show the results for one of the H-P cases we have considered before, i.e. $E_{HH} = -1,\ E_{HP} = -1/\sqrt{2}$ and $E_{PP} = 0$. Table 4.2.a corresponds to the case where we have fixed both the variance and the average of the E's, leaving only one parameter to be determined. Table 4.2.b shows the results when only the variance of the interaction parameters is fixed to set the energy scale, and two parameters are left to be determined, as in our method.

With the parameters obtained from the minimization we checked how many of the good sequences of the PDB still have their unique ground state in the correct conformation among all the possible conformations obtained by exact enumeration. In contrast to our method, not all of the sequences in the PDB find the correct native state, as can be seen in the tables. Note that neither the success rate, nor the values of the potentials are monotonic as a function of the chain length, at least within the small range of lengths used.

Table 4.2.c shows the results for the 4 amino acid case for the same four sets of potential parameters used to test our method. The variance and the average potential have been fixed to their exact values (thus there are 8 free parameters and not 9 as in our case). For one parameter set we have also implemented the MS optimization method. MS use the following expressions for $\langle E \rangle$ and var(E) (see the discussion following eq.13):

$$\langle E \rangle = \sum_{i<j} P_{\mu_i,\mu_j} \langle \Delta_{i,j} \rangle \tag{14}$$

$$\text{var}(E) = \sum_{i<j} \sum_{k<l} P_{\mu_i,\mu_j} P_{\mu_k,\mu_l} T_{ij,kl} \tag{15}$$

with

$$\langle \Delta_{i,j} \rangle = \frac{n}{n_{tot}} \tag{16}$$

and

$$T_{ij,kl} = \begin{cases} \frac{1}{n_{tot}^2} & (i,j) \neq (k,l) \\ \\ \frac{1}{n_{tot}} - \frac{1}{n_{tot}^2} & (i,j) = (k,l) \end{cases} \tag{17}$$

where n is the number of contacts in the native conformation, n_{tot} is the total number of the topologically possible contacts and the indices $i, j, \ldots$ run from 1 to the length of the chain (14 in our case). Using the MS hypothesis, we found a different expression for $T_{ij,kl}$:

$$T_{ij,kl} = \begin{cases} \frac{n(n-1)}{n_{tot}(n_{tot}-1)} - \frac{n^2}{n_{tot}^2} & (i,j) \neq (k,l) \\ \\ \frac{n}{n_{tot}} - \frac{n^2}{n_{tot}^2} & (i,j) = (k,l) \end{cases} \tag{18}$$

The results corresponding to both assignments, (18) and (17), are also reported in table 4.2.c and should be compared with the results of our method in table 3.2 . Figures 4. a, b, c and d are the analogs of fig. 2. a, b, c and d for the MS method. Figure 4.a shows the extracted potentials using both the exact $\langle E \rangle$ and var(E) and the approximation (16) and (18) (which according to table 4.2.c works better than the MS one, i.e. (16) and (17)) for parameter set 1.

parameter set	$\#_{tot}$	$\#_{wrong}$	success
1	628	64	89.8 %
2	716	80	88.2 %
3	840	14	98 %
4	798	96	88 %
1 (using eq 18)	628	71	88 %
1 (using eq 17)	628	105	83 %

(4.2.c)

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figure captions

Fig.1 The 2 dimensional cells for the target parameters ($E_{HH} = -1, E_{PP} = 0, E_{HP} = 0$) (in units of k_BT), using all the structures as alternatives, for different sequence lengths. Indicated are the cell (shaded area), the target parameters (fat point), the sequence length (10, 12, 14, 16), the volume (if the cell is closed) or the opening angle alpha of the allowed area.

Fig.2 Derived potential versus true potential for the 4 aminoacid problem (in units of k_BT). Results for the parameter set 1 (a), 2 (b), 3 (c) and 4 (d).

Fig.3 Effect of the PDB size on the success rate using the extracted parameters to determine the ground state configurations for new sets of sequences, for different minimum energy gaps Δ (in units of k_BT). For the case with $\Delta > 2$ (open triangles), the cell is not closed for small N. When the cell is closed, the success rate is almost 100

Fig.4 Derived potential versus true potential (in units of k_BT), for the 4 aminoacid problem using the MS method. Results for the parameter set 1 (a), 2 (b), 3 (c) and 4 (d). Fig. 1.a also shows the results using the approximation (16) and (18) (open circles).

table captions

Tab. 3.1.a,3.1.b,3.1.c Results for the H-P model fixing E_{HH} to its true value to set the energy scale (in units of k_BT). The table shows the sequence length, the obtained interaction parameters, the true minimum gap with the target parameters, the obtained mxm-gap, the volume of the cell (or opening angle in cases in which the cell is not closed) both using all conformations and only the "good" ones as alternative conformations. The success rate in the prediction of native conformations of the "good" sequences with the obtained parameters is 100% in all cases that the cell is closed.

Tab. 3.2 Results for the 4 aminoacid model. For each parameter set, the table shows the size of the PDB used for the derivation of the potential and the success rate in the correct prediction of the native state for each of the training set sequences.

Tab. 4.1 Summary of the results of TD [14] using the Miyazawa-Jernigan scheme [6], for a sequence length of 14 monomers. The table shows the true parameters, the parameters obtained by TD and the success rate in the prediction of native conformations of the "good" sequences with the obtained parameters.

Tab. 4.2.a Results for the H-P model, using the Z-score of MS [15], fixing both the variance and the average of the interaction parameters to their true values. The table displays the sequence length, the derived interaction parameters, the total number of good sequences, the number of sequences for which the predicted ground state is wrong, and the success rate.

Tab. 4.2.b Same as Tab. 4.2.a, but only fixing the variance of the interaction parameters.

Tab. 4.2.c Results for the 4 aminoacid model, using the MS method [15], fixing both the variance and the average of the interaction parameters to their true values. The table shows the identification of the parameter set, the size of the PDB used for the derivation of the potentials, the number of failures in the prediction of the correct native state, and the success rate. In the top 4 lines, the exact $\langle E \rangle$ and $\mathrm{var}(E)$ are used, whereas for the fifth line the approximation (16) and (18), and for the sixth line the approximation (16) and (17) are used.

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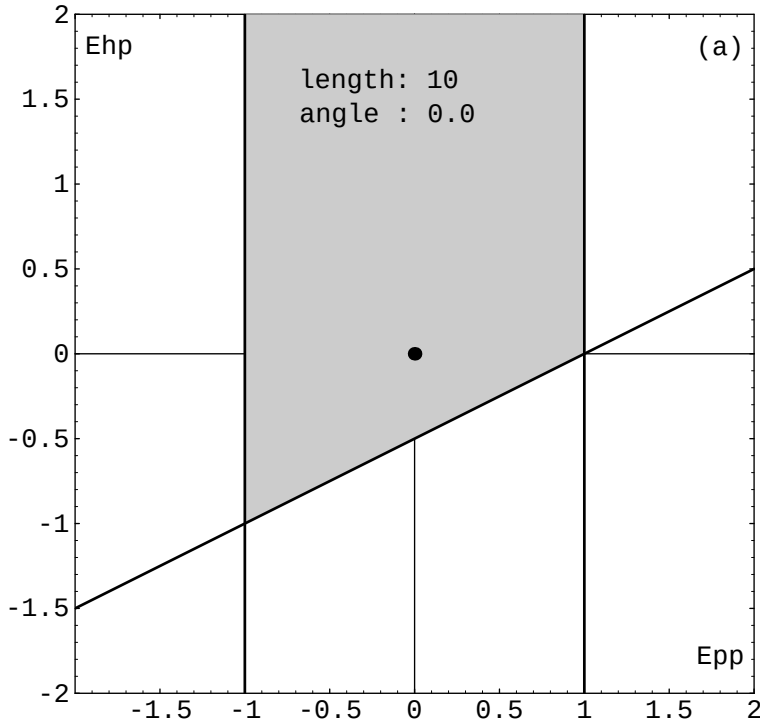
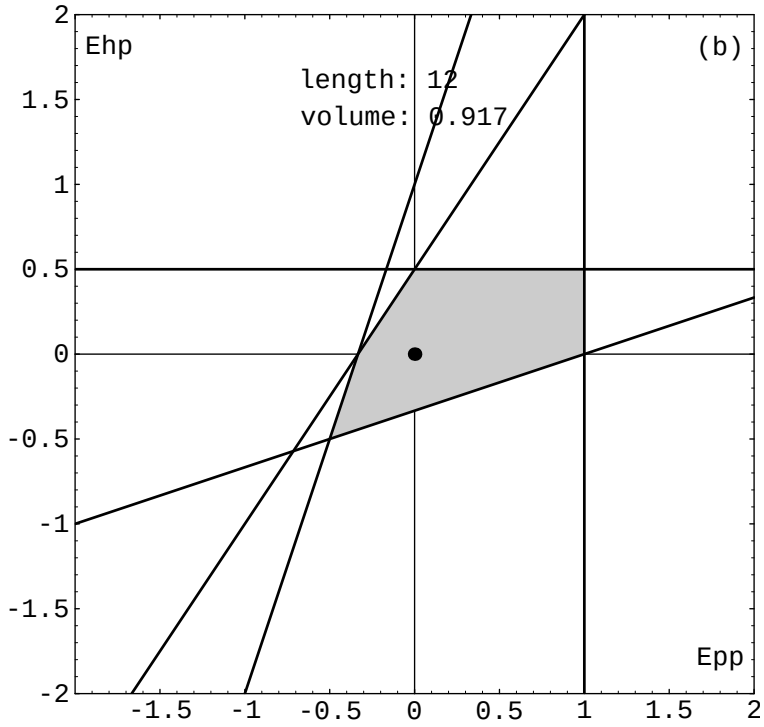
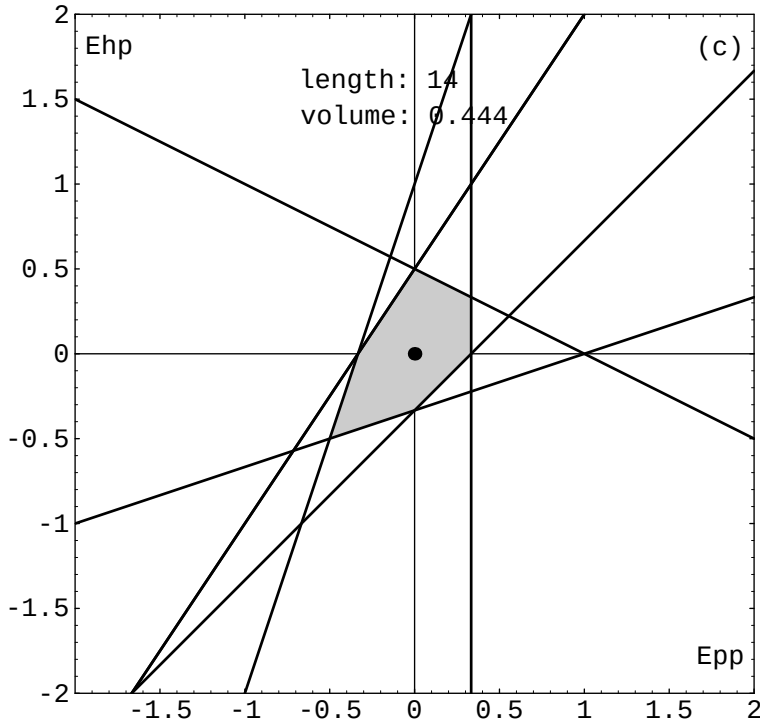

Ehp
(a)
length: 10
angle : 0.0
Epp
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2


Ehp
(b)
length: 12
volume: 0.917
Epp
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2


Ehp
(c)
length: 14
volume: 0.444
Epp
2
1.5
1
0.5
0
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-1
-1.5
-2
-1.5
-1
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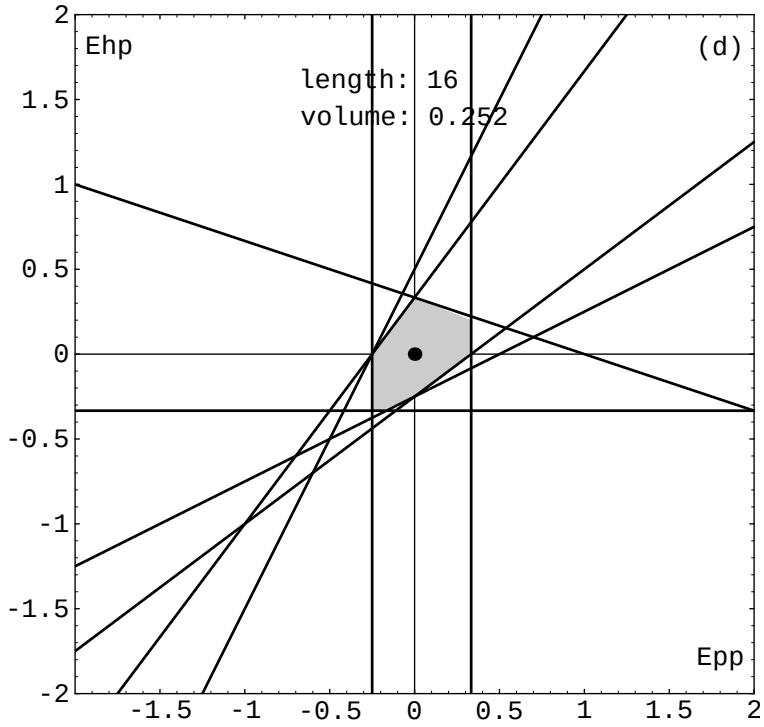
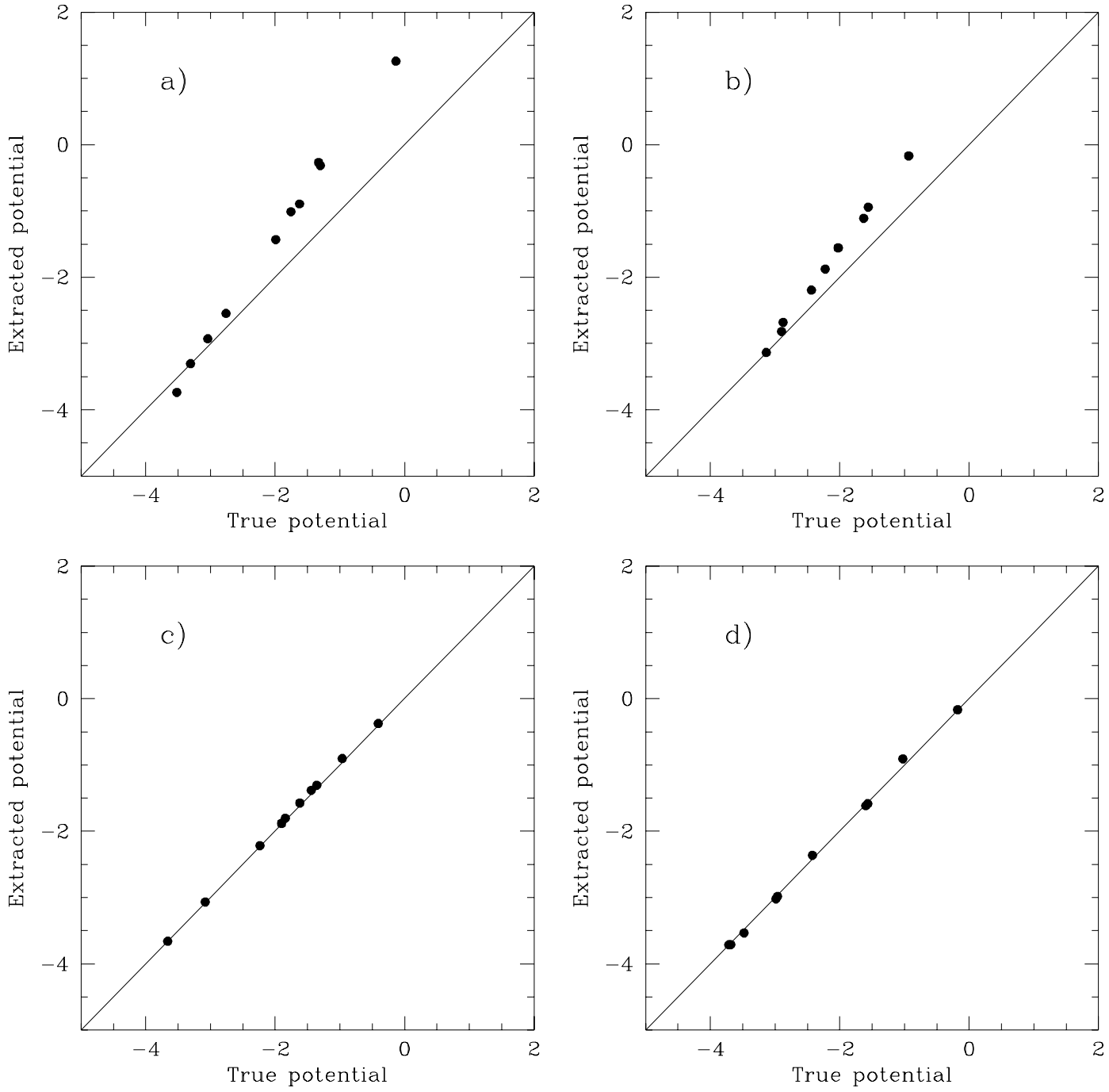
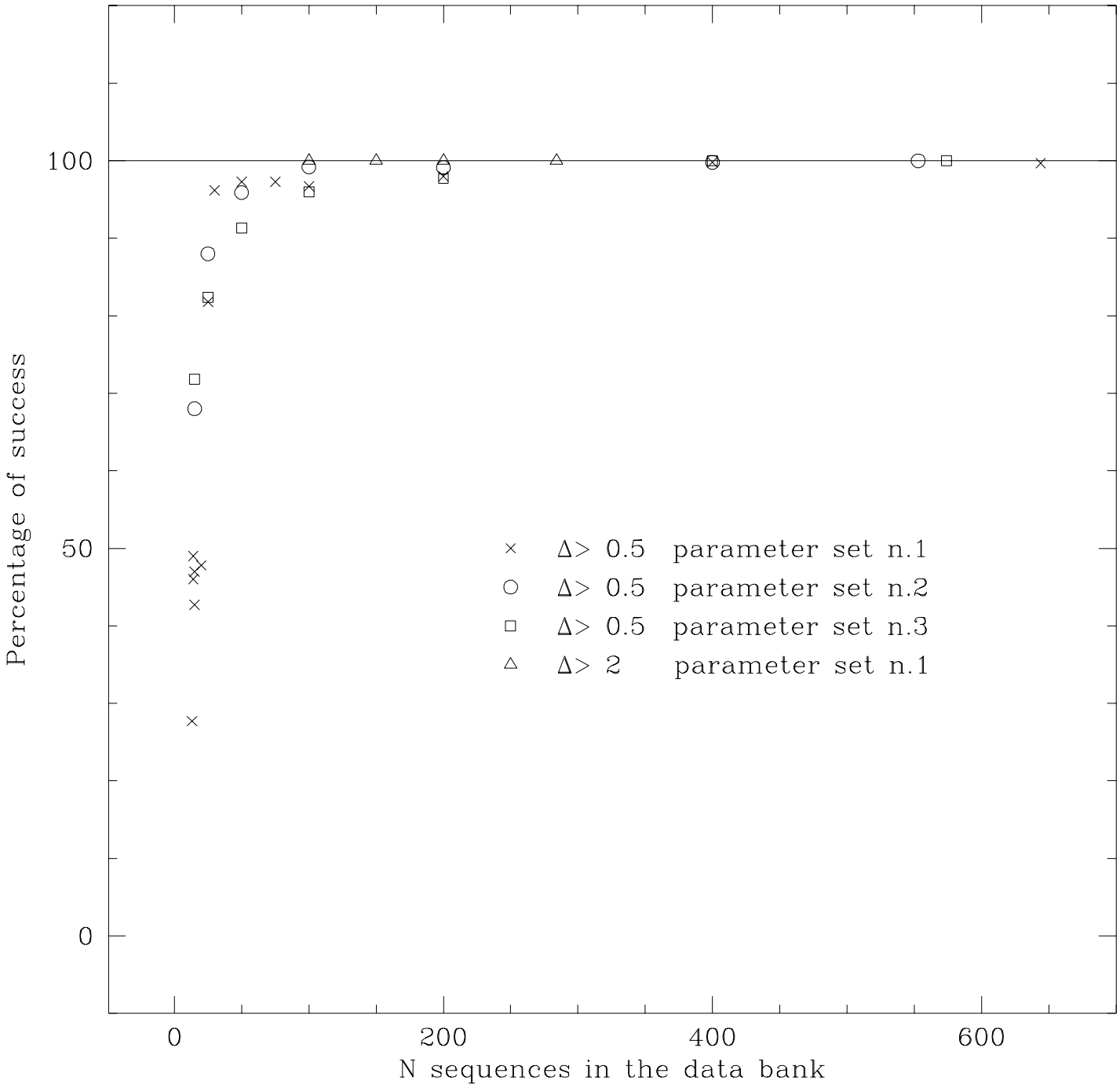
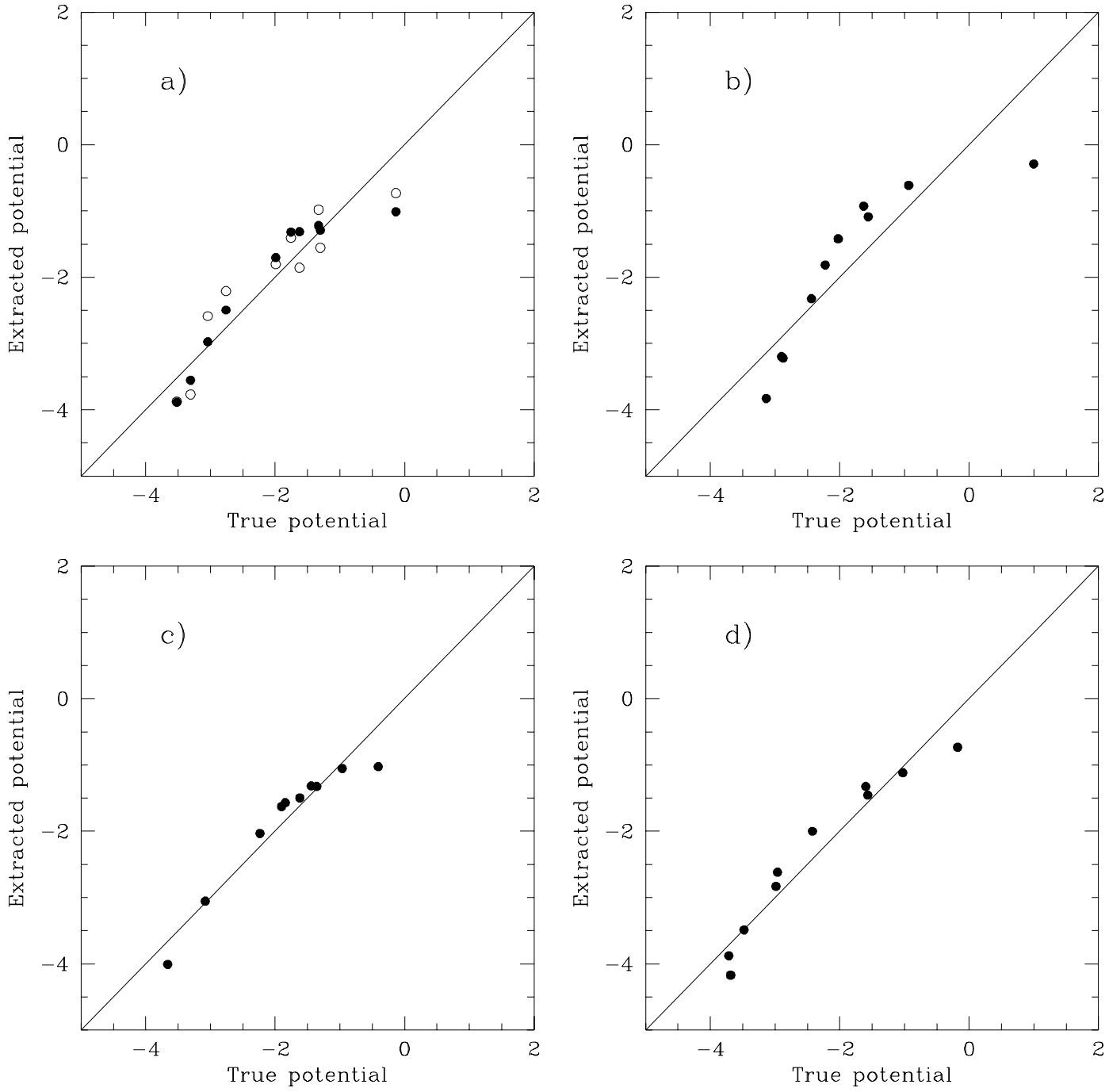
0
0.5
1
1.5
2


Ehp
(d)
length: 16
volume: 0.252
Epp
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2


a)
b)
c)
d)
Extracted potential
True potential
2
0
−2
−4
−4
−2
0
2


100
50
0
Percentage of success
0
200
400
600
N sequences in the data bank
Δ> 0.5 parameter set n.1
Δ> 0.5 parameter set n.2
Δ> 0.5 parameter set n.3
Δ> 2 parameter set n.1


a)
b)
c)
d)
Extracted potential
True potential
2
0
−2
−4
−4
−2
0
2